

## Taxonomic Revision of *Raorchestes menglaensis* (Kou, 1990) (Amphibia: Anura), with Descriptions of Two New Species from Yunnan, China

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**Abstract** *Raorchestes* is a group of Bush frogs mainly distributed in South and Southeast Asia that are poorly recognized by morphological criteria. We recognize the true *R. menglaensis* (Kou) based on 10 specimens newly collected from the type locality. An expanded description is also provided, and we delist the recently reported record, *R. parvulus* (Boulenger) from China. Combing with morphological and molecular data, two new species are respectively described from Menghai and Lvchun, Yunnan, China. *Raorchestes hillisi* sp. nov. is distinguished from all other congeners by the following combination of morphological characters: (1) small body sized (males 15.9–17.7 mm,  $n = 7$ ; female 17.5 mm,  $n = 1$ ); (2) head longer than wide; (3) snout longer than eye diameter; (4) the tip of upper jaws slightly notched; (5) internasal space slightly less than interorbital space; (6) tympanum distinct; (7) fingers lacking lateral dermal fringe; (8) outside of toe I and both sides of toe II lacking lateral dermal fringe, other toes having weak lateral dermal fringes; (9) rudimentary web on toes; (10) lacking a series of tubercles along the outer sides of forearm and foot; (11) discs of fingers and toes not orange. *Raorchestes huanglianshan* sp. nov. is distinguished from all other congeners by the following combination of

morphological characters: (1) small body size (males 17.0–19.6 mm,  $n = 12$ ; female 21.5 mm,  $n = 1$ ); (2) head slightly wider than long or equal; (3) snout longer than eye diameter; (4) the tip of upper jaws slightly notched; (5) internasal space distinctly less than interorbital space (6) tympanum distinct; (7) fingers lacking lateral dermal fringe; (8) toes lacking lateral dermal fringe; (9) rudimentary web on toes; (10) lacking a series of tubercles along the outer sides of forearm and foot; (11) discs of all fingers and toes orange or parts of them orange in life.

**Keywords** bush frog, distribution, *Raorchestes hillisi* sp. nov., *Raorchestes huanglianshan* sp. nov., *Raorchestes parvulus*, revision, taxonomy

### 1. Introduction

The Bush frogs in the genus *Raorchestes* Biju, Shouche, Dubois, Dutta, and Bossuyt, 2010 are largely nocturnal species, having small body size (SVL 15 to 45 mm) and large transparent gular pouch while calling, lacking vomerine teeth, which are holding direct development (Biju *et al.*, 2010). Sixty-two species are known in *Raorchestes* and mainly distributed in South and Southeast Asia, including southern India to Nepal, Myanmar, Thailand, Laos, southern China, Vietnam, and West Malaysia. More than 80% known species distributed in Western Ghats of southern India (Frost, 2020).

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Received: 18 February 2020 Accepted: 10 April 2020

Four species, *R. longchuanensis* (Yang and Li, 1979), *R. menglaensis* (Kou, 1990), *R. parvulus* (Boulenger, 1893), and *R. cangyuanensis* Wu, Suwannapoon, Xu, Murphy, and Che, 2019 are currently recorded from China (Wu *et al.*, 2019; Yu *et al.*, 2019; AmphibiaChina, 2020). However, *Raorchestes* species were barely understood from China, for example, both *R. menglaensis* and *R. longchuanensis* were only known from their original descriptions decades ago (Yang and Li, 1979; Kou, 1990). No detailed morphological, phylogenetic, and ecological data reported, many *Raorchestes* species still remain poorly diagnosed, and the limited available information hampered the identification of these small-sized tree frogs.

In addition, the taxonomic status of *R. menglaensis* seems to be questionable. Originally described by Kou (1990) from “Zhushihe, Mengla County, Yunnan Province”, China, the morphology of *R. menglaensis* has been known from type series and a single male specimen only (Fei *et al.*, 2009). Moreover, although the phylogenetic position of *R. menglaensis* was provided by Li *et al.* (2009) and Yu *et al.* (2009) based on the same specimen (060821286Rao) from “Lvchun, Yunnan”, it cannot represent true *R. menglaensis* from type locality. With the lack of true *R. parvulus*, Yu *et al.* (2019) recently reported *R. parvulus* as a new country record to China based on specimens collected from Mengla County, however, the locality of their new record is close to the type locality of *R. menglaensis*. Given the conservative evolution of the morphology and high frequency of variation in the genus *Raorchestes* (Orlov *et al.*, 2012; Yu *et al.*, 2019), the taxonomic identity of *R. menglaensis* is needed to be clarified.

In the present study, the phylogenetic relationships among *Raorchestes* species were reconstructed, including all species and some unidentified populations from China and southeastern Asia, and the samples of *R. menglaensis*, *R. longchuanensis*, and *R. cangyuanensis* were from their type localities. We confirmed the phylogenetic position and morphology of true *R. menglaensis*, and both the phylogenetic and morphological evidence revealed that the record of *R. 'parvulus'* from China by Yu *et al.* (2019) should be misidentification of *R. menglaensis*. In addition, populations respectively from Menghai, southern Yunnan and Lvchun, southeastern Yunnan formed two distinct clades, which represent two new species, and we describe them based on molecular and morphological differences herein.

## 2. Materials and Methods

**2.1. Sampling** A total of 38 specimens were collected from Yunnan, China, including 10 specimens (9 males, one female) from Zhushihe, Mengla; six specimens (five males, one female) from Menglun, Mengla; eight specimens (seven males, one female) from Xiding, Menghai; 14 specimens (13 males, one

female) from Mt. Huanglian, Lvchun. Following euthanasia, liver tissues were taken and preserved in 95% ethanol, and the specimen was fixed in 10% formalin solution and was transferred to 75% ethanol after fieldwork. All specimens including types were deposited in the Herpetological Museum, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, China.

**2.2. Morphological data** Morphological comparisons: All measurements were made with slide calipers to the nearest 0.1 mm. Morphological characters used and their measurement methods followed Fei *et al.* (2009), webbing formula followed Savage and Heyer (1997). The morphological characters and their abbreviations as: SVL, snout-vent length; HL, head length; HW, head width; SL, snout length; INS, internarial distance; IOS, interorbital distance; EHD, eye horizontal diameter; UEW, maximum width of upper eyelid; TD, tympanum diameter; FAHL, forelimb and hand length; LAW, width of lower arm; HAL, hand length; FML, femur length; TBL, tibia length; TFL, length of tarsus and foot; FOL, foot length.

Morphological data of congeners were obtained from vouchered specimens (Appendix) as well as from literatures (Kou, 1990; Boulenger, 1893; Smith, 1924; Bossuyt and Dubois, 2001; Fei *et al.*, 2009).

**2.3. Molecular data and analyses** Genomic DNA was extracted from liver tissue preserved in 95% ethanol using an Ezup Column Animal Genomic DNA Purification Kit (Sangon Biotech, China). For each specimen in this study, the mitochondrial gene 16S ribosomal RNA (16S rRNA) gene was sequenced. The fragments of 16S rRNA were amplified using primers 16Sar-L (5'-CGCCTGTTTATCAAAACAT-3') and 16Sbr-H (5'-CCGGTCTGAACTCAGATCACGT-3') (Vijayakumar *et al.*, 2014). Polymerase chain reactions (PCR) amplifications were performed in a 25  $\mu$ l reaction volume with an initial denaturation at 94 °C for 3 min, followed by 40 cycles of 94 °C for 1 min, 51 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 10 min. The PCR products were purified and then sequenced in both forward and reverse directions using an ABI 3730XL sequencer (Applied Biosystems, Foster City, CA, USA) by Sangon Biotech Co., Ltd (Chengdu, China). All sequences were submitted and deposited in GenBank (Table 1). Additional 68 sequences of *Raorchestes* species and three outgroup taxa were obtained from GenBank; outgroup taxa, *Beddomixalus bijui* (Zachariah, Dinesh, Radhakrishnan, Kunhikrishnan, Palot, and Vishnudas), *Nasutixalus medogensis* (Jiang, Wang, Yan, and Che), and *Pseudophilautus asankai* (Manamendra-Arachchi and Pethiyagoda), were selected based on previous phylogenetic studies (Jiang *et al.*, 2016; Chan *et al.*, 2018).

The dataset was aligned and edited using MEGA 6.0 with

**Table 1** Information on Voucher No., GenBank No., and localities of specimens used in this study, collections and their abbreviations see material and methods.

Species	Voucher No.	GenBank No.	Locality	Resource
<i>Raorchestes menglaensis</i>	CIB 116338	MT488403	China: Zhushihe, Mengla, Yunnan	This study
<i>Raorchestes menglaensis</i>	CIB 116340	MT488404	China: Zhushihe, Mengla, Yunnan	This study
<i>Raorchestes menglaensis</i>	CIB 116341	MT488405	China: Zhushihe, Mengla, Yunnan	This study
<i>Raorchestes menglaensis</i>	CIB 116343	MT488406	China: Zhushihe, Mengla, Yunnan	This study
<i>Raorchestes menglaensis</i>	CIB 116344	MT488407	China: Zhushihe, Mengla, Yunnan	This study
<i>Raorchestes menglaensis</i>	CIB 116347	MT488408	China: Menglun, Mengla, Yunnan	This study
<i>Raorchestes menglaensis</i>	CIB 116348	MT488409	China: Menglun, Mengla, Yunnan	This study
<i>Raorchestes menglaensis</i>	CIB 116349	MT488410	China: Menglun, Mengla, Yunnan	This study
<i>Raorchestes hillisi</i>	CIB 116331	MT488411	China: Xiding, Menghai, Yunnan	This study
<i>Raorchestes hillisi</i>	CIB 116329	MT488412	China: Xiding, Menghai, Yunnan	This study
<i>Raorchestes hillisi</i>	CIB 116330	MT488413	China: Xiding, Menghai, Yunnan	This study
<i>Raorchestes huanglianshan</i>	CIB 116365	MT488414	China: Mt. Huanglian, Lvchun, Yunnan	This study
<i>Raorchestes huanglianshan</i>	CIB 116353	MT488415	China: Mt. Huanglian, Lvchun, Yunnan	This study
<i>Raorchestes huanglianshan</i>	CIB 116281	MT488416	China: Mt. Huanglian, Lvchun, Yunnan	This study
<i>Raorchestes huanglianshan</i>	CIB 116354	MT488417	China: Mt. Huanglian, Lvchun, Yunnan	This study
<i>Raorchestes huanglianshan</i>	CIB 116357	MT488418	China: Mt. Huanglian, Lvchun, Yunnan	This study
<i>Raorchestes huanglianshan</i>	CIB 116358	MT488419	China: Mt. Huanglian, Lvchun, Yunnan	This study
<i>Raorchestes agasthyaensis</i>	CESF 492	JX092723	-	Vijayakumar et al., 2014
<i>Raorchestes akroparallagi</i>	CESF 061	JX092650	-	Vijayakumar et al., 2014
<i>Raorchestes anili</i>	CESF 386	JX092708	-	Vijayakumar et al., 2014
<i>Raorchestes aureus</i>	CESF 1164	KM596540	India: Elivalmalai Massif, Western Ghats	Vijayakumar et al., 2014
<i>Raorchestes beddomii</i>	CESF 072	JX092653	-	Vijayakumar et al., 2014
<i>Raorchestes bobingeri</i>	CESF 1238	JX092680	-	Vijayakumar et al., 2014
<i>Raorchestes bombayensis</i>	CESF 1010	JX092657	-	Vijayakumar et al., 2014
<i>Raorchestes cangyuanensis</i>	KIZ 015855	MN475866	China: Cangyuan, Yunnan	Wu et al., 2019
<i>Raorchestes cangyuanensis</i>	KIZ 015856	MN475867	China: Cangyuan, Yunnan	Wu et al., 2019
<i>Raorchestes cangyuanensis</i>	KIZ 015857	MN475868	China: Cangyuan, Yunnan	Wu et al., 2019
<i>Raorchestes charius</i>	CESF 132	JX092691	-	Vijayakumar et al., 2014
<i>Raorchestes chlorosomma</i>	BNHS 4426	EU450017	India: Munnar, Idukki, Kerala	Biju and Bossuyt, 2009
<i>Raorchestes chotta</i>	CESF 1003	JX092656	-	Vijayakumar et al., 2014
<i>Raorchestes chromasynchysi</i>	CESF 1127	JX092667	-	Vijayakumar et al., 2014
<i>Raorchestes coonoorensis</i>	CESF 439	JX092716	-	Vijayakumar et al., 2014
<i>Raorchestes crustai</i>	CESF 1199	JX092677	-	Vijayakumar et al., 2014
<i>Raorchestes dubois</i>	CESF 114	JX092668	-	Vijayakumar et al., 2014
<i>Raorchestes emeraldi</i>	CESF 1365	KM596556	India: Valparai Plateau, Anaimalai Massif	Vijayakumar et al., 2014
<i>Raorchestes flaviocularis</i>	CESF 1252	KM596549	-	Vijayakumar et al., 2014
<i>Raorchestes ghatei</i>	AGCZRL-Amphibia-128	KF366391	India: Thoseghar, Satara, Maharashtra	Padhye et al., 2013
<i>Raorchestes glandulosus</i>	CESF 1080	JX092665	-	Vijayakumar et al., 2014
<i>Raorchestes graminirupes</i>	CESF 044	JX092649	-	Vijayakumar et al., 2014
<i>Raorchestes griet</i>	CESF 073	JX092654	-	Vijayakumar et al., 2014
<i>Raorchestes gryllus</i>	ROM 30288	GQ285674	Vietnam: Tuyen Quang, Pac Ban	Li et al., 2009
<i>Raorchestes gryllus</i>	ROM 30298	MN475869	Vietnam: Tam Dao, Vinh Phu Province	Wu et al., 2019
<i>Raorchestes indigo</i>	CESF 138	KM596557	-	Vijayakumar et al., 2014
<i>Raorchestes jayarami</i>	CESF 1260	JX092686	-	Vijayakumar et al., 2014
<i>Raorchestes johnceei</i>	CESF 1236	JX092679	-	Vijayakumar et al., 2014
<i>Raorchestes kadalarensis</i>	CESF 1766	JX092701	-	Vijayakumar et al., 2014
<i>Raorchestes kaikatti</i>	CESF 444	JX092718	-	Vijayakumar et al., 2014
<i>Raorchestes kakachi</i>	CESF 1385	KM596558	-	Vijayakumar et al., 2014
<i>Raorchestes longchuanensis</i>	5Rao	GQ285675	China: Longchuan, Yunnan	Li et al., 2009

(Continued Table 1)

Species	Voucher No.	GenBank No.	Locality	Resource
<i>Raorchestes longchuanensis</i>	KIZ 048468	MN475870	China: Gongdong, Longchuan, Yunnan	Wu <i>et al.</i> , 2019
<i>Raorchestes longchuanensis</i>	KIZ 048492	MN475871	China: Gongdong, Longchuan, Yunnan	Wu <i>et al.</i> , 2019
<i>Raorchestes luteolus</i>	CESF 1012	JX092659	-	Vijayakumar <i>et al.</i> , 2014
<i>Raorchestes manohari</i>	CESF 1187	JX092674	-	Vijayakumar <i>et al.</i> , 2014
<i>Raorchestes marki</i>	CESF 467	JX092719	-	Vijayakumar <i>et al.</i> , 2014
<i>Raorchestes 'menglaensis'</i>	060821286Rao	GQ285676	China: Lvchun, Yunnan	Li <i>et al.</i> , 2009
<i>Raorchestes montanus</i>	CESF 130	KM596552	-	Vijayakumar <i>et al.</i> , 2014
<i>Raorchestes munnarensis</i>	CESF 094	JX092655	-	Vijayakumar <i>et al.</i> , 2014
<i>Raorchestes nerostagona</i>	CESF 1061	JX092661	-	Vijayakumar <i>et al.</i> , 2014
<i>Raorchestes ochlandrae</i>	CESF 1111	JX092666	-	Vijayakumar <i>et al.</i> , 2014
<i>Raorchestes 'parvulus'</i>	K3056	KR828034	Thailand: Doi Chiang Dao, Chiangmai	Grosjean <i>et al.</i> , 2015
<i>Raorchestes cf. parvulus</i>	LSUHC 11118	MH590201	Malaysia: Gunung Stong, Kelantan	Chan <i>et al.</i> , 2018
<i>Raorchestes cf. parvulus</i>	LSUHC 7460	MH590205	Cambodia	Chan <i>et al.</i> , 2018
<i>Raorchestes cf. parvulus</i>	LSUHC 10473	MH590203	Malaysia: Gunung Jerai, Kedah	Chan <i>et al.</i> , 2018
<i>Raorchestes cf. parvulus</i>	LSUHC 5880	MH590204	Malaysia: Gunung Jerai, Kedah	Chan <i>et al.</i> , 2018
<i>Raorchestes cf. parvulus</i>	LSUHC 7596	MH590202	Malaysia: Pulau Langkawi, Kedah	Chan <i>et al.</i> , 2018
<i>Raorchestes 'parvulus'</i>	KUHE 19128	AB871421	Thailand: Doi Inthanon	Nguyen <i>et al.</i> , 2014
<i>Raorchestes cf. parvulus</i>	KUHE 38322	LC012865	Thailand: Khao Sabap	Nguyen <i>et al.</i> , 2015
<i>Raorchestes cf. parvulus</i>	K3148/2006.2572	KR828035	Laos: Louangphrabang, Houey Thao	Grosjean <i>et al.</i> , 2015
<i>Raorchestes cf. parvulus</i>	0069Y	KR828036	Thailand: Phitsanulok, Lan Son	Grosjean <i>et al.</i> , 2015
<i>Raorchestes 'parvulus'</i>	KIZ 20160365	MK564629	China: Menglun, Mengla, Yunnan	Yu <i>et al.</i> , 2019
<i>Raorchestes 'parvulus'</i>	KIZ 20160366	MK564630	China: Menglun, Mengla, Yunnan	Yu <i>et al.</i> , 2019
<i>Raorchestes 'parvulus'</i>	KIZ 20160367	MK564631	China: Menglun, Mengla, Yunnan	Yu <i>et al.</i> , 2019
<i>Raorchestes 'parvulus'</i>	KIZ 20160372	MK564632	China: Menglun, Mengla, Yunnan	Yu <i>et al.</i> , 2019
<i>Raorchestes 'parvulus'</i>	KIZ 20160373	MK564633	China: Menglun, Mengla, Yunnan	Yu <i>et al.</i> , 2019
<i>Raorchestes 'parvulus'</i>	KIZ 20160374	MK564634	China: Menglun, Mengla, Yunnan	Yu <i>et al.</i> , 2019
<i>Raorchestes ponmudi</i>	CESF 063	JX092651	-	Vijayakumar <i>et al.</i> , 2014
<i>Raorchestes primarrumpfi</i>	CESF 442	KM596575	-	Vijayakumar <i>et al.</i> , 2014
<i>Raorchestes resplendens</i>	CESF 1258	JX092683	-	Vijayakumar <i>et al.</i> , 2014
<i>Raorchestes signatus</i>	-	AY141841	-	Meegaskumbura <i>et al.</i> , 2002
<i>Raorchestes sushili</i>	CESF 1259	JX092684	-	Vijayakumar <i>et al.</i> , 2014
<i>Raorchestes theuerkaufi</i>	CESF 1342	JX092693	-	Vijayakumar <i>et al.</i> , 2014
<i>Raorchestes tinniens</i>	CESF 438	JX092715	-	Vijayakumar <i>et al.</i> , 2014
<i>Raorchestes travancoricus</i>	CESF 473	JX092721	-	Vijayakumar <i>et al.</i> , 2014
<i>Raorchestes tuberohumerus</i>	CESF 148	JX092697	-	Vijayakumar <i>et al.</i> , 2014
<i>Raorchestes uthamani</i>	CESF 483	JX092722	-	Vijayakumar <i>et al.</i> , 2014
<i>Beddomixalus bijui</i>	TNHM (H) 12.6.18/57	KC594290	India: Arippa, Kollam district, Kerala	Abraham <i>et al.</i> , 2013
<i>Nasutixalus medogensis</i>	KIZ 016395	KU243082	China: Medog, Tibet	Jiang <i>et al.</i> , 2016
<i>Pseudophilautus asankai</i>	WHT5107	FJ788160	-	Meegaskumbura <i>et al.</i> , 2009

default settings (Tamura *et al.*, 2013), and the alignment was checked by eye and adjusted manually. Bayesian inference (BI) analyses were implemented using MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003). The best-fit model was selected in PartitionFinder 2 (Lanfear *et al.*, 2017) under Akaike Information Criterion (AIC), which is a GTR+I+G model for all the three codon positions. Four Markov chains were executed and the data set was run for 10 million generations to allow

adequate time for convergence, sampling the Markov chains at intervals of 100 generations. Bayesian posterior probability (BPP) was determined to test the confidence of tree topology, nodes in the trees were considered strongly supported when BPP  $\geq 0.95$ . Convergence was investigated in Tracer 1.6 (Rambaut *et al.*, 2013), and the first 25% trees were discarded as burn-in. Maximum likelihood (ML) were conducted in RAxML 8.2.10 (Stamatakis, 2014) under the best-fit model of evolution based

on the AIC (GTRGAMMA) criterion. Tree searches were performed 100 times and bootstrap proportions (BSP) were assessed using the rapid-bootstrapping algorithm (1000 non-parametric bootstrap replicates) to test the node support, where nodes with  $BSP \geq 70$  were significantly supported. Uncorrected pairwise distances ( $p$ -distances) of the 16s dataset among species of targeted *Raorchestes* taxa were calculated using MEGA 6 (Tamura *et al.*, 2013).

**2.4. Abbreviations** CIB, the Herpetological Museum, Chengdu Institute of Biology, Chinese Academy of Sciences; KIZ, Kunming Institute of Zoology, Chinese Academy of Sciences; CESF, Centre for Ecological Sciences-Frogs, Indian Institute of Science; BNHS, Bombay Natural History Society; AGCZRL, Abasaheb Garware College-Zoology Research Laboratory; ROM, Royal Ontario Museum; Rao, Dr. Dingqi Rao's lab collections in KIZ; KUHE, Graduate School of Human and Environmental Studies, Kyoto University; TNHM (H), Trivandrum Natural History Museum; LSUHC, La Sierra University Herpetological Collection; WHT, Wildlife Heritage Trust of Sri Lanka. K and Y, unknown resource.

### 3. Results

**3.1. Phylogenetic analyses** Phylogenetic analyses showed that our newly collected *Raorchestes* specimens were recovered as three major clades (Figure 1):

i) the first clade from Menghai, Yunnan, China formed a distinct clade with strong support (Figure 1, clade B;  $BSP = 100$ ;  $BPP = 1.00$ ), which represents a new species, *Raorchestes hillisi* sp. nov., as described herein, individuals within clade B possessed no genetic divergence;

ii) the second clade from Mt. Huanglian, Lvchun, Yunnan, China, and together with *Raorchestes 'menglaensis'* (060821286Rao, from Lvchun, Yunnan, China) (Li *et al.*, 2009) formed a monophyletic clade with short internal branch lengths (clade C;  $BSP = 98$ ;  $BPP = 1.00$ ), which represents another new species, *Raorchestes huanglianshan* sp. nov., the uncorrected genetic  $p$ -distances varied from 0–1.74% within clade C;

iii) the third clade included specimens from Zhushihe, Mengla, Yunnan, China (type locality of *R. menglaensis*), Menglun, Mengla and some specimens stated to be *Raorchestes 'parvulus'* by Yu *et al.* (2019) (clade D;  $BSP = 54$ ), the uncorrected genetic  $p$ -distances varied from 0–0.25% within clade D (Table 2).

Although the true *R. menglaensis* (as *R. menglaensis* *sensu stricto*) seemed to be nested within the *R. parvulus* lineages, *R. parvulus* was recovered as paraphyletic, which were recovered as several highly diverged, strongly supported, monophyletic matrilines that correspond with geographic distribution. However, we cannot evaluate the taxonomic statuses of

these unexamined clades but only assess the phylogenetic position of *R. menglaensis* *sensu stricto* (see below). Based on both morphological and genetic data obtained from topotypes of *R. menglaensis*, we provided a re-description and revised previous misidentifications of this highly variable species herein.

### 3.2. Morphological comparisons

i) our newly collected specimens from Mengla, Yunnan are similar to the original description of *R. menglaensis* in having: head longer than wide; snout longer than eye diameter; fingers and toes lacking lateral dermal fringe; discs of fingers and toes not orange.

ii) *R. menglaensis* differs from *R. parvulus* in having: fingers and toes lacking lateral dermal fringes.

iii) morphological descriptions provided by Yu *et al.* (2019) differ from *R. parvulus* in having: fingers and toes lacking lateral dermal fringes.

### 3.3. Systematic account

#### Re-description of *Raorchestes menglaensis* (Kou) (Figures 2, 3)

*Philautus menglaensis* Kou, 1990, in Zhao (ed.), From Water onto Land: 210. Type locality: Zhushihe, Mengla, Yunnan, China; alt. 900 m. Holotype: (YU) A845090, ♂, by original designation.

*Philautus (Philautus) menglaensis*: Bossuyt and Dubois, 2001, Zeylanica, 6: 59.

*Aquixalus menglaensis*: Fei, Hu, Ye, and Huang, 2009, Fauna Sinica, Amph. 2: 722.

*Raorchestes menglaensis*: Biju, Shouche, Dubois, Dutta, and Bossuyt, 2010, Curr. Sci., Bangalore, 98: 1120, by implication.

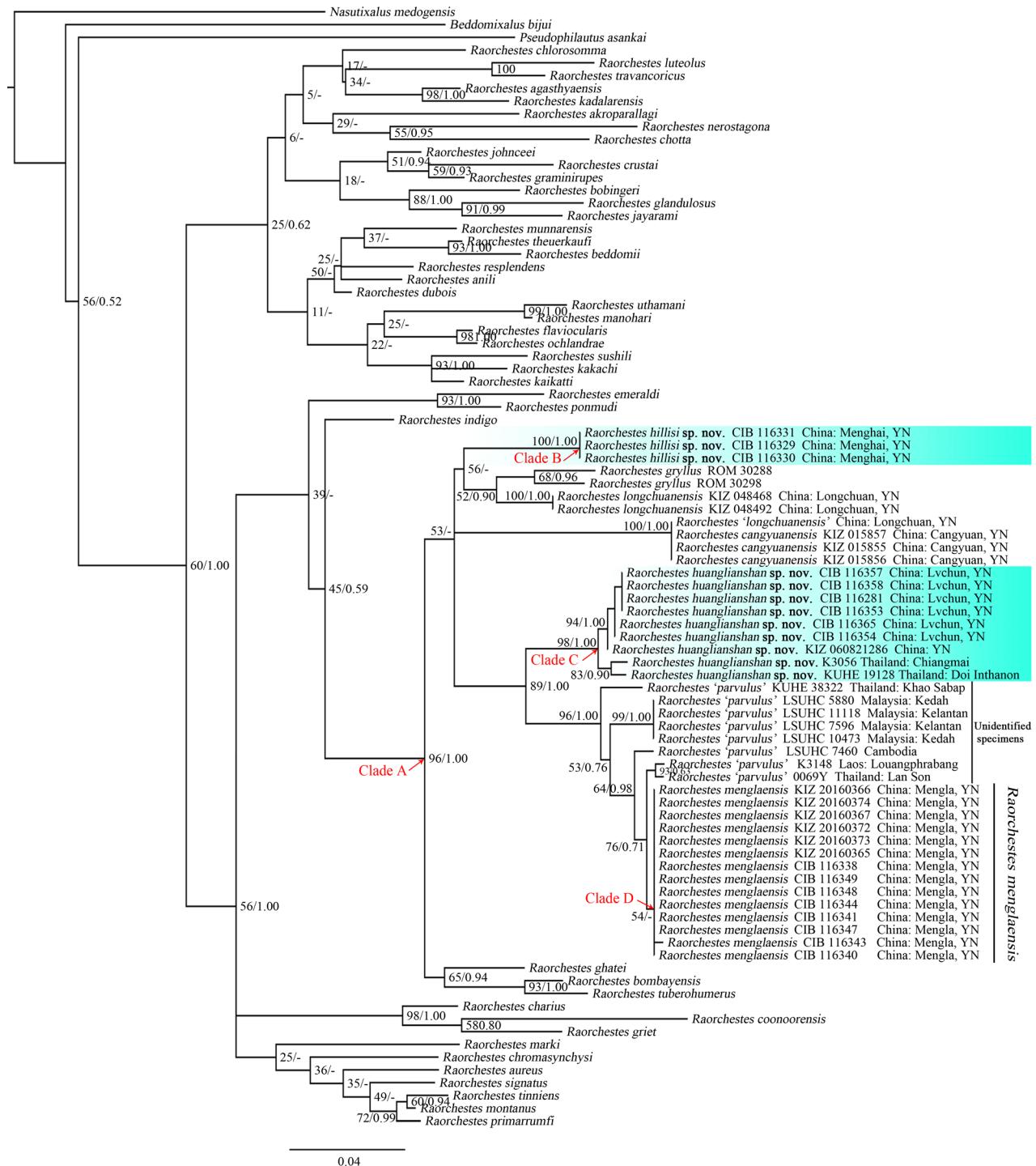
*Lixixalus menglaensis*: Fei, Ye, and Jiang, 2012, Colored Atlas Chinese Amph. Distr.: 513.

*Raorchestes parvulus*: Yu, Liu, Hou, Li, and Yang, 2019, Zootaxa, 4577 (2): 386.

**Referred specimens:** nine male specimens and one female specimen from Zhushihe (type locality), Mengla, Yunnan; five male specimens and one female specimen from Menglun, Mengla, Yunnan. Specimens collected by Ke JIANG, Junfeng GUO, Wenbo YU, Yonghong LIU, Zhongxiong FU, Hanliu AI.

**Remark:** the specific epithet is named after its type locality Mengla (Yunnan, China). We suggest “Mengla Bush Frog” as its English common name, and “Meng La Guan Shu Wa (勐腊灌树蛙)” as its Chinese common name.

**Diagnosis:** (1) relatively small body sized (males 16.6–21.6 mm,  $n = 15$ ; female 18.9–20.5 mm,  $n = 2$ ); (2) head longer than wide; (3) snout longer than eye diameter; (4) the tip of upper jaws slightly notched; (5) internasal space sub-equal to interorbital space; (6) tympanum distinct; (7) fingers lacking lateral dermal fringe; (8) toes lacking lateral dermal fringe; (9) rudimentary web on toes; (10) lacking a series of tubercles along the outer sides of forearm



**Figure 1** BI phylogenetic tree estimated from mtDNA sequences depicting phylogenetic relationships of *Raorchestes*, supportive values are showed above branches as BPP/BSP, values in parentheses after clade labels represent uncorrected *p*-distances of the corresponding clades.

and foot; (ii) discs of fingers and toes not orange in life.

**Description:** small body sized (males 16.6–21.6 mm,  $n = 15$ ; female 18.9–20.5 mm,  $n = 2$ ). Head longer than wide; snout rounded, upper jaw protruding slightly in ventral view; the tip of upper jaws slightly notched; canthus rostralis distinct; loreal region concave; snout slightly longer than eye diameter; nostrils

oval, lacking flap, closer to snout tip than to eye; internasal space near equal to interorbital space; interorbital space 1.50 times larger than upper eyelid width; tongue notched at the tip; lingual papillae absent; vomerine teeth absent; tympanum distinct; supratympanic fold distinct; eyes small, pupil horizontal.

**Table 2** Uncorrected pairwise sequence divergence (%) among 16S ribosomal RNA mtDNA sequences of clade A, including clade B, C, and D, as shown in phylogenetic tree (see Figure 1); \* represents new taxa described in this study.

Clade	Taxa	Voucher	Locality	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A D <i>R. menglaensis</i>	CIB 116338	China	1																								
A D <i>R. menglaensis</i>	CIB 116347	China	2	0.00																							
A D <i>R. menglaensis</i>	KIZ 20160372	China	3	0.00	0.00																						
A D <i>R. menglaensis</i>	KIZ 20160373	China	4	0.00	0.00	0.00																					
A D <i>R. menglaensis</i>	CIB 116343	China	5	0.25	0.25	0.25	0.25																				
A - <i>R. 'parvulus'</i>	0069Y	Thailand	6	0.75	0.75	0.75	0.75	0.75	1.23																		
A - <i>R. 'parvulus'</i>	2006.2572	Laos	7	0.77	0.77	0.77	0.77	0.77	1.27	0.26																	
A - <i>R. 'parvulus'</i>	LSUHC 7460	Cambodia	8	1.00	1.00	1.00	1.00	1.00	1.53	1.50	1.50	1.54															
A - <i>R. 'parvulus'</i>	LSUHC 7596	Malaysia	9	1.96	1.96	1.96	1.96	1.96	2.80	2.74	2.81	2.19															
A - <i>R. 'parvulus'</i>	KUHE 38322	Thailand	10	2.30	2.30	2.30	2.30	2.30	3.06	2.99	3.08	1.60	2.35														
A - <i>R. bombayensis</i>	CESF 1010	India?	11	4.34	4.24	4.41	4.41	4.83	5.75	5.90	4.80	5.30	5.58														
A - <i>R. longhuaniensis</i>	KIZ 048468	China	12	4.97	4.86	5.06	5.06	5.38	6.78	7.22	5.68	5.18	5.65	5.05													
A C R. <i>huanglianshan</i> *	CIB 116365	China	13	4.90	4.79	4.98	4.98	5.36	5.74	5.88	4.79	4.71	4.41	6.09													
A C R. <i>huanglianshan</i> *	KIZ 060821286	China	14	4.70	4.60	4.78	4.78	5.09	5.47	5.61	4.58	4.50	4.21	5.89	5.41	0.18											
A C R. <i>huanglianshan</i> *	CIB 116357	China	15	5.08	4.97	5.17	5.17	5.61	5.99	6.14	4.99	4.90	4.61	6.27	5.78	0.18	0.37										
A C R. <i>huanglianshan</i> *	KUHE 19128	Thailand	16	5.14	5.14	5.14	5.14	5.36	5.74	5.88	4.79	4.74	4.55	6.34	6.01	1.18	0.99	1.38									
A C R. <i>huanglianshan</i> *	K3056	Thailand	17	5.99	5.99	5.99	5.99	6.46	5.74	6.14	5.75	5.49	5.50	8.00	6.52	1.49	1.24	1.74	1.00								
A - <i>R. gryllus</i>	ROM 30298	Vietnam	18	5.13	5.02	5.22	5.22	5.60	6.48	6.65	6.05	6.53	6.20	4.83	3.17	5.95	5.75	6.13	6.39	7.23							
A - <i>R. gryllus</i>	ROM 30288	Vietnam	19	5.95	5.95	5.95	5.95	5.87	6.98	7.16	6.80	6.88	6.92	5.37	3.50	6.51	6.32	6.70	6.72	7.21	2.71						
A - <i>R. tuberohumerus</i>	CESF 148	India?	20	5.22	5.10	5.31	5.31	5.53	6.72	6.63	5.75	6.05	6.11	2.08	5.34	6.23	6.04	6.42	6.49	8.25	4.76	5.30					
A B R. <i>hillisi</i> *	CIB 116331	China	21	5.85	5.72	5.95	5.95	6.39	6.75	6.92	5.99	6.88	6.35	4.98	4.66	5.71	5.52	5.89	6.52	6.98	3.72	4.98	5.09	0.00			
A B R. <i>hillisi</i> *	CIB 116329	China	22	5.85	5.72	5.95	5.95	6.39	6.75	6.92	5.99	6.88	6.35	4.98	4.66	5.71	5.52	5.89	6.52	6.98	3.72	4.98	5.09	0.00			
A - <i>R. cangyuanensis</i>	KIZ 015855	China	23	6.12	6.17	6.23	6.23	6.92	7.54	7.73	7.10	7.17	7.41	6.74	5.98	6.53	6.34	6.72	7.21	8.77	5.60	5.84	6.50	6.17	6.17		
A - <i>R. ghati</i>	AGCZRL 128	India	24	6.10	5.96	6.21	6.21	6.87	7.31	7.26	5.44	5.75	6.22	4.43	4.44	5.59	5.38	5.78	5.39	6.28	4.80	5.42	3.55	5.01	5.01	6.00	



**Figure 2** Photographs of different individuals of *Raorchestes menglaensis* from the type locality, Zhushihe, Mengla, Yunnan, showing (A) dorsal view and (B) ventral view of a male; (C) to (F) dorsal view of four males. Photographs by Wenbo YU.

Forelimb length near equal to half of body length; fingers lacking lateral dermal fringe and web; subarticular tubercles prominent (fingers: I = 1, II = 1, III = 2, IV = 1); relative lengths of fingers: I < II < IV < III; discs rounded; circummarginal grooves present on all fingers; nuptial pad present on the dorsal surface of first finger.

Hindlimbs short, heels just touch when folded at right angles to the body; tibiotarsal articulation reaching the eye when adpressed; supernumerary tubercles and subarticular tubercle present (toes: I = 1, II = 1, III = 2, IV = 3, V = 2); relative lengths of toes: I < II < III < V < IV; disks rounded, the size of disks equal to these on fingers; circummarginal grooves present

on all toes; no web between toe I and toe II, web rudimentary between other toes, webbing formula: II 1-2 III 1-2<sup>1/2</sup> IV 2<sup>1/2</sup>-1 V; toes lacking lateral dermal fringe; subarticular tubercles prominent, rounded; inner metatarsal tubercle distinct, oval, outer metatarsal tubercle absent.

Skin on dorsal surface smooth, few small tubercles scattered on shoulder and towards the vent. Distinct tubercles scattered along the temporal region and lateral body. Ventral surface covered with relatively large flat tubercles.

**Coloration in life:** dorsal color pale brown or dark brown, with a dark triangular blotch on interorbital space, two or more patches of yellow pigmentation on dorsal head; a "X" shaped dark brown or black blotch on back, from posterior eyes to each side of lumbar, sometimes indistinct; a dark brown or black blotch on each crotch; dorsal limbs with more or less distinct dark brown or black bars; dorsal surface usually covered by small white warts; some individuals having a pale line along the middle of back and hind limbs; dorsal side of discs pale brown or yellow. Ventral sides of head and body grayish-white, with small dark gray spots, the tubercles white; ventral side of limbs gray. Iris brown.

**Coloration in preservative:** dorsal color changed to gray, the blotches or spots brownish-gray, warts white. Ventral color white, spots brownish-gray.

**Sexual dimorphism:** in males, white nuptial pad present on the dorsal surface of the first finger; a large external single subgular vocal sac present (vocal sac of some individuals not expanded), a pair of slit-like openings near the inner corners of mouth; white lineae masculinae visible on ventral body.

**Variation:** The measurements are given in Table 3 and 4, and the variation are as follows: Vocal sac of four specimens (CIB116337-38, CIB116340, CIB116343) from type locality and five specimens from Menglun (CIB116347-51) are distinctly expended, other five specimens (CIB116339, CIB116341-42, CIB116344-45) from type locality are not expanded.

**Distribution:** known from Zhushihe (type locality) (Figure 4) and Menglun, Yunnan, China; based on the phylogenetic relationships, it is probably also from Lan Son, Thailand; Louangphrabang, Laos; and Cambodia.

**Habitat:** this species was found calling on bushes near evergreen broad-leaved forest at night, at altitude from 520 m to 903 m. *Megophrys parva* (Boulenger), *Leptobrachium chapaense* (Bourret), *Limnonectes liui* (Yang), *Polypedates impresus* Yang, and *Thelodera albopunctatum* (Liu and Hu) were found.

***Raorchestes hillisi* sp. nov. Jiang, Ren, Guo, Wang, and Li** (Figures 5, 6, 7).

**Holotype:** CIB 116329, an adult male (field No. BN2018006),



**Figure 3** Photographs of a male individual of *Raorchestes menglaensis* from Menglun, Mengla, Yunnan, showing (A) dorsal view; (B) ventral view. Photographs by Jinlong REN.

collected from Xiding (21.935607°N, 100.149527°E, altitude 1719 m), Menghai, Yunnan, China on 10 May 2018 by Ke JIANG.

**Paratypes:** six adult males (CIB 116330-35) and an adult female CIB 116336, as same locality and date as holotype, collected by Ke JIANG, Junfeng GUO, Lingui He, Kai WANG and Zhongxiong FU.

**Etymology:** the specific epithet "hillisi" is named after Dr. David M. Hillis (University of Texas at Austin), for his excellent contributions to the evolutionary studies, and we acknowledge his support to us. We suggest "Hillis's Bush Frog" as its English common name, and "Meng Hai Guan Shu Wa (勐海灌树蛙)" as its Chinese common name.

**Diagnosis:** this new species is identified to the genus *Raorchestes* by its molecular phylogenetic position and the following morphological characters: (1) having small body size (SVL 15.0 to 45.0 mm); (2) large gular pouch transparent while calling; (3) lacking vomerine teeth.

***Raorchestes hillisi* sp. nov.** is distinguished from all other

**Table 3** Measurements of *Raorchestes menglaensis* from type locality Zhushih, Mengla, Yunnan (in mm).

Number	CIB 116337	CIB 116338	CIB 116339	CIB 116340	CIB 116341	CIB 116342	CIB 116343	CIB 116344	CIB 116345	Range	Mean ± S.D.	Ratio to SVL	CIB 116346	Ratio to SVL
Sex	♂	♂	♂	♂	♂	♂	♂	♂	♂			♀		
SVL	19.7	19.6	18.2	18.0	18.3	17.1	21.6	19.5	16.6	16.6–21.6	18.7 ± 1.5		18.9	
HL	7.7	7.9	7.2	7.1	7.2	6.8	7.9	7.2	6.7	6.7–7.9	7.3 ± 0.4	39.2%	7.8	41.1%
HW	7.4	7.8	6.9	6.8	6.9	6.3	7.4	7.2	6.5	6.3–7.8	7.0 ± 0.5	37.7%	6.9	36.7%
SL	3.0	2.8	3.1	2.6	2.8	2.6	3.1	2.7	2.7	2.6–3.1	2.8 ± 0.2	15.1%	2.7	14.0%
INS	2.1	2.2	2.2	2.0	2.1	2.0	2.3	2.1	1.9	1.9–2.3	2.1 ± 0.1	11.3%	2.1	11.3%
IOS	2.4	2.8	2.5	2.4	2.4	2.4	3.0	2.3	2.3	2.0–3.0	2.4 ± 0.2	13.1%	2.6	13.6%
UEW	1.5	1.5	1.5	1.6	1.7	1.7	1.9	1.6	1.5	1.5–1.9	1.6 ± 0.1	8.7%	1.9	10.1%
EHD	2.6	2.6	2.3	2.5	2.7	2.4	2.9	2.6	2.6	2.3–2.9	2.6 ± 0.2	13.8%	2.6	13.5%
TD	0.9	0.7	0.9	0.7	0.7	0.7	0.7	0.8	0.7	0.7–0.9	0.8 ± 0.1	3.7%	0.7	3.8%
FAHL	10.2	10.3	9.6	9.3	9.4	8.6	10.7	9.6	8.4	8.4–10.7	9.6 ± 0.7	51.2%	9.0	47.8%
LAW	1.5	1.7	1.6	1.6	1.6	1.6	1.9	1.5	1.5	1.5–1.9	1.6 ± 0.1	8.8%	1.2	6.3%
HAL	5.8	5.8	5.2	5.3	5.1	5.2	6.1	5.6	4.5	4.5–6.1	5.4 ± 0.5	28.9%	5.1	27.2%
FML	9.6	9.5	9.0	8.7	8.9	8.0	8.8	8.7	7.8	7.8–9.6	8.8 ± 0.6	47.0%	9.0	47.9%
TBL	9.6	9.8	9.1	9.0	9.2	8.3	9.6	9.4	8.2	8.2–9.8	9.1 ± 0.6	49.0%	9.2	48.5%
TFL	12.6	13.0	12.0	11.8	12.2	10.7	13.3	12.3	10.6	10.6–13.3	12.1 ± 0.9	64.8%	11.8	62.6%
FOL	7.5	7.9	7.0	7.3	7.1	6.3	8.4	7.2	5.9	5.9–8.4	7.2 ± 0.7	38.6%	7.1	37.7%

**Table 4** Measurements of *Raorchestes menglaensis* from Menglun, Mengla, Yunnan (in mm).

Number	CIB 116347	CIB 116348	CIB 116349	CIB 116350	CIB 116351	Range	Mean ± S.D.	Ratio to SVL	CIB 116352	Ratio to SVL
Sex	♂	♂	♂	♂	♂			♀		
SVL	19.3	19.6	18.4	19.4	19.9	19.3–19.9	19.3 ± 0.6		20.5	
HL	7.6	7.8	7.6	7.7	7.8	7.6–7.8	7.7 ± 0.1	39.8%	8.7	42.3%
HW	6.9	7.5	6.4	7.5	7.7	6.4–7.7	7.2 ± 0.5	37.1%	8.3	40.4%
SL	2.7	2.7	2.7	2.9	2.9	2.7–2.9	2.8 ± 0.1	14.5%	3.2	15.6%
INS	1.9	2.2	1.8	2.1	2.1	1.9–2.2	2.0 ± 0.2	10.4%	2.5	12.0%
IOS	2.1	2.4	2.1	2.6	2.5	2.1–2.6	2.3 ± 0.2	12.0%	2.5	12.4%
UEW	1.7	1.5	1.7	1.8	2.0	1.5–2.0	1.7 ± 0.2	8.8%	2.1	10.0%
EHD	2.5	2.6	2.3	2.6	2.7	2.3–2.7	2.5 ± 0.1	13.1%	2.9	13.9%
TD	0.8	0.8	0.8	0.8	0.8	0.8	0.8 ± 0.0	4.2%	0.9	4.5%
FAHL	9.7	10.5	9.6	10.3	9.8	9.6–10.5	10.0 ± 0.4	51.5%	10.7	52.2%
LAW	1.6	1.9	1.7	1.5	1.6	1.5–1.9	1.7 ± 0.1	8.6%	1.5	7.3%
HAL	5.4	6.3	4.9	5.8	5.8	4.9–6.3	5.6 ± 0.5	29.1%	6.3	30.7%
FML	9.5	9.7	9.3	9.9	9.2	9.2–9.9	9.5 ± 0.3	49.1%	9.9	48.2%
TBL	9.5	10.3	9.3	10.0	9.3	9.3–10.3	9.7 ± 0.4	49.9%	10.8	52.7%
TFL	12.6	13.2	12.2	13.8	12.3	12.2–13.8	12.8 ± 0.7	66.2%	13.6	66.0%
FOL	7.9	7.9	7.2	8.5	7.9	7.2–8.5	7.9 ± 0.5	40.7%	8.1	39.4%

congeners by the following combination of morphological characters: (1) small body sized (males 15.9–17.7 mm,  $n = 7$ ; female 17.5 mm,  $n = 1$ ); (2) head longer than wide; (3) snout longer than eye diameter; (4) the tip of upper jaws slightly notched; (5) internasal space sub-equal to interorbital space; (6) tympanum distinct; (7) fingers lacking lateral dermal fringe; (8) outside of toe I and both sides of toe II lacking lateral dermal fringe, other toes having weak lateral dermal fringes; (9) rudimentary web on toes; (10) lacking a series of tubercles along the outer sides of

forearm and foot; (11) discs of fingers and toes not orange in life.

**Description of holotype:** small body size (SVL = 17.3 mm). Head longer than wide (HL/HW = 1.12); snout rounded, upper jaw protruding slightly in ventral view; the tip of upper jaws slightly notched; canthus rostralis distinct; loreal region concave; snout slightly longer eye diameter (SL/ED = 1.13); nostrils oval, lacking flap, closer to snout tip than to eye; internasal space near equal to interorbital space (IOS/INS = 1.05); interorbital space 1.40 times larger than upper eyelid width (IOS = 2.1 mm; UEW

= 1.4 mm); tongue notched at the tip; lingual papillae absent; vomerine teeth absent; tympanum distinct; supratympanic fold distinct; eyes small, pupil horizontal; a large external single subgular vocal sac present, a pair of slit-like openings near the inner corners of mouth.

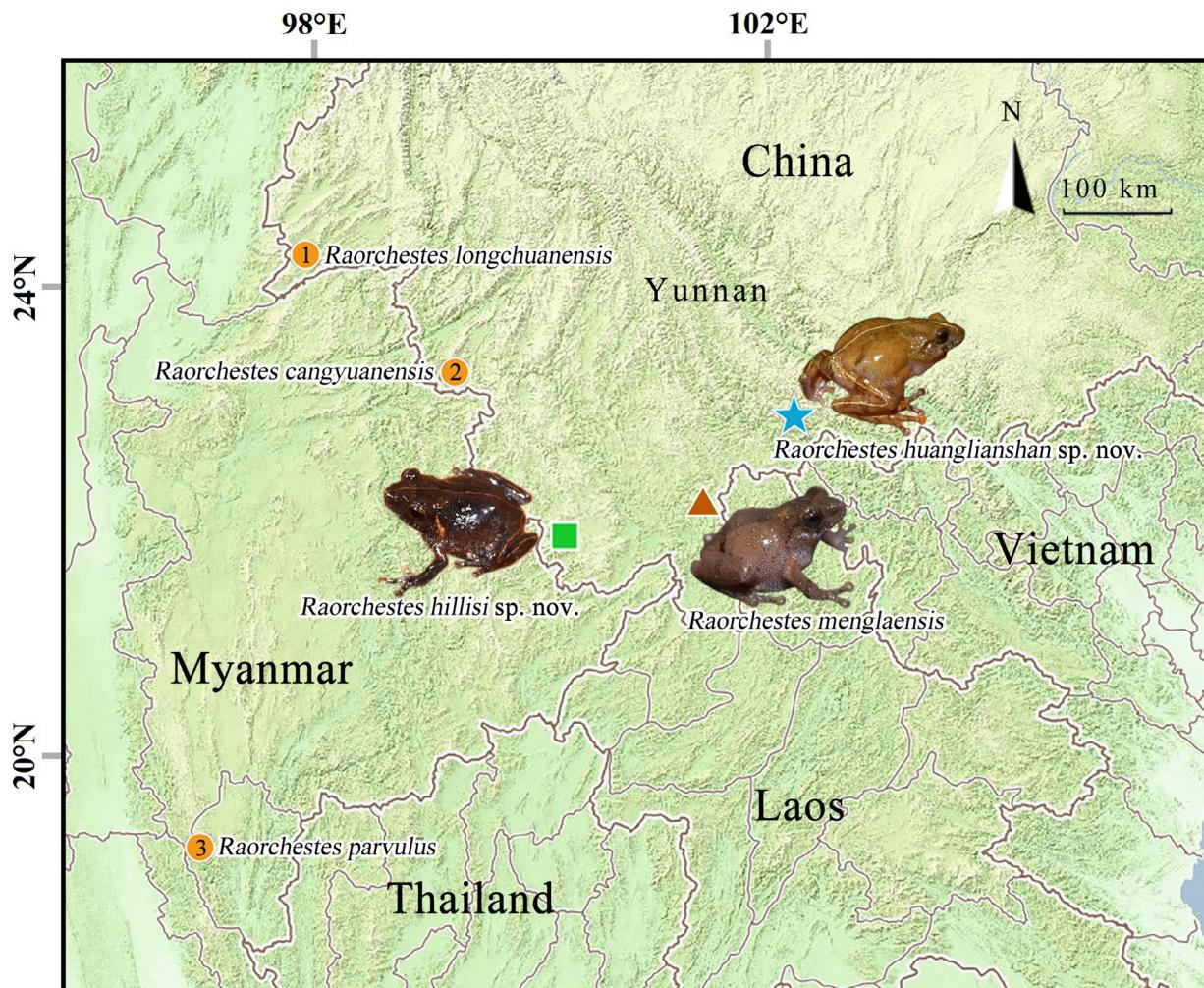
Forelimb length near equal to half of body length (FAHL/SVL = 0.52); fingers lacking lateral dermal fringe and web; subarticular tubercles prominent (fingers: I = 1, II = 1, III = 2, IV = 1); relative lengths of fingers: I < II < IV < III; discs rounded; circummarginal grooves present on all fingers; nuptial pad present on the dorsal surface of first finger.

Hindlimbs short, heels just touch when folded at right angles to the body (TBL = 8.3 mm, FML = 8.5 mm); tibiotarsal articulation reaching the eye when adpressed; supernumerary tubercles and subarticular tubercle present (toes: I = 1, II = 1, III = 2, IV = 3, V = 2); relative lengths of toes: I < II < III < V <

IV; disks rounded, the size of disks equal to these on fingers; circummarginal grooves present on all toes; no web between toe I and toe II, web rudimentary between other toes, webbing formula: II 1-2 III 1-2<sup>1/2</sup> IV 2<sup>1/2</sup>-1 V; outside of toe I and both sides of toe II lacking lateral dermal fringe, other toes having weak lateral dermal fringe; subarticular tubercles prominent, rounded; inner metatarsal tubercle distinct, oval, outer metatarsal tubercle absent.

Skin on dorsal surface smooth, few indistinct tubercles scattered on shoulder and towards the vent. Distinct tubercles scattered along the temporal region and lateral body. ventral surface covered with relatively large flat tubercles.

**Coloration in life:** dorsal and lateral sides brown, with a dark brown triangular blotch on interorbital space, one patch of yellowish-brown pigmentation on forehead, two patches of yellowish-brown pigmentation on dorsal head; an indistinct



**Figure 4** The type localities of *Raorchestes* species related to this study, *Raorchestes menglaensis* (brown triangle), *Raorchestes hillisi* sp. nov. (green square), *Raorchestes huanglianshan* sp. nov. (blue star), *R. longchuanensis* (orange circle, No. 1), *R. cangyuanensis* (orange circle, No. 2), and *R. parvulus* (orange circle, No. 3).

")(" shaped dark brown blotch on fore part of back; an distinct dark brown blotch on each crotch; dorsal limbs with more or less distinct dark brown bars; dorsal surface without white warts; dorsal side of discs yellowish-brown. A dark brown stripe under the supratympanic fold, the posterior end of jaws and upper arm with yellowish-brown pigmentations. Ventral sides of head and body grayish-white, with small dark gray spots, the tubercles white; ventral side of limbs gray, with white spots. Iris brown.

**Coloration in preservative:** dorsal color changed to grayish-brown, the blotches or spots blackish-brown; yellowish-brown pigmentations changed to pale brown. Ventral color whitish-gray, spots grayish-brown.

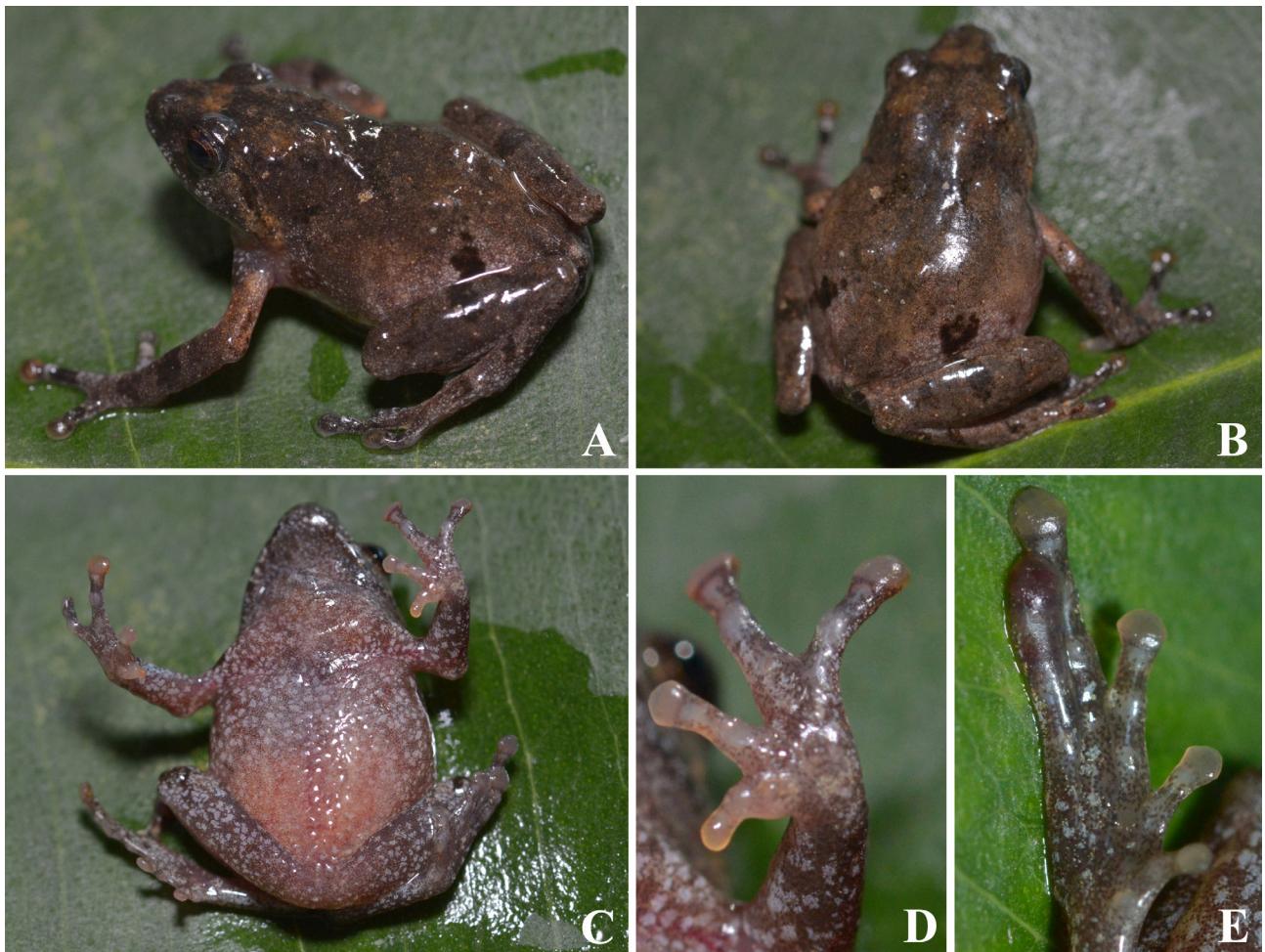
**Variation:** The measurements are given in Table 5 and the variation are as follows: CIB 116334 having a pale line along the middle of back and hind limb; three males (CIB 116332–33, 115335) having lighter ground color on dorsal side.

**Sexual dimorphism:** in males, white nuptial pad present on the dorsal surface of the first finger; a large external single subgular vocal sac present, extended to the chest, a pair of slit-like openings near the inner corners of mouth; white lineae masculinae visible on ventral body.

**Distribution:** currently known only from the type locality, Xiding (Figure 4), Menghai, Yunnan.

**Habitat:** this new species was found in bushes surrounded by moist subtropical secondary evergreen broad-leaved forest (Figure 8) near Xiding Village at night (10 pm. to 12 pm.) on 10 May 2018, the altitude is 1719 m a. s. l., the weather is light rain. All male individuals were found on the bush leaves while calling, eggs were not observed. *Polypedates impresus* and *Microhyla fissipes* Boulenger were also found in bushes, and *Pseudocalotes microlepis* (Boulenger) was found on trees near the bushes.

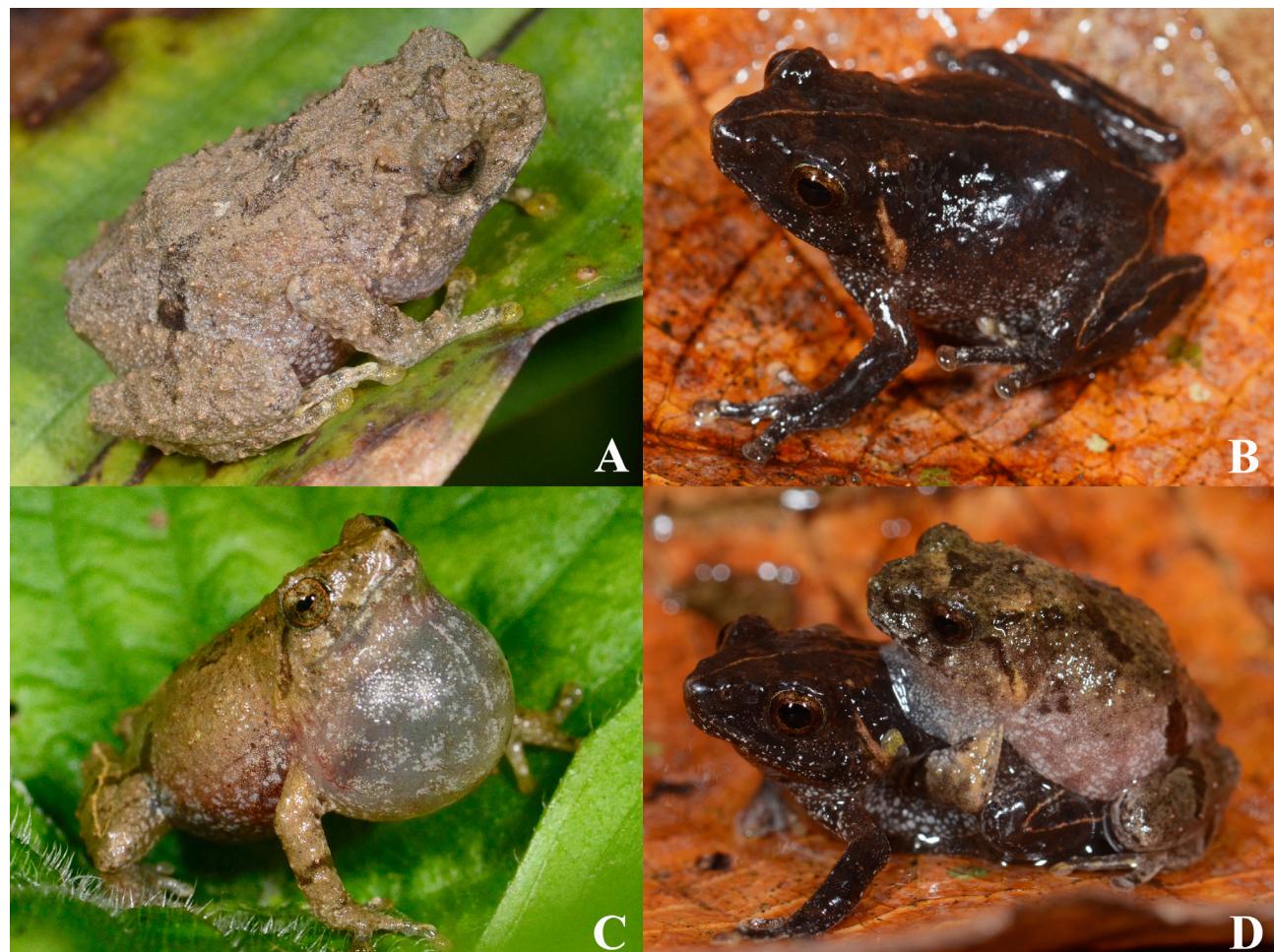
**Comparison:** only five species of the genus *Raorchestes* known

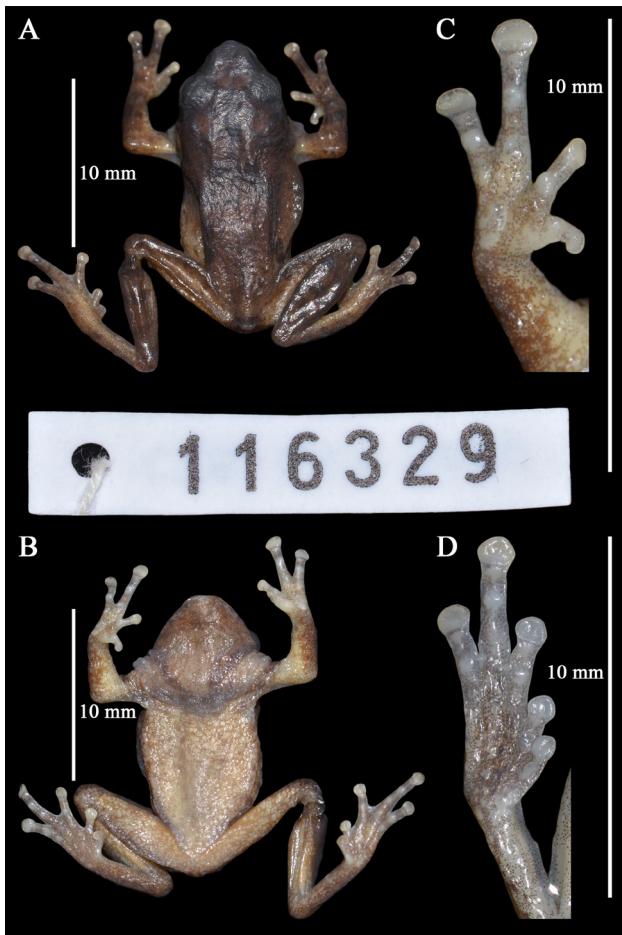


**Figure 5** Photographs of the holotype of *Raorchestes hillisi* sp. nov. in life from the type locality, Xiding, Menghai, Yunnan, showing (A) dorsolateral view; (B) dorsal view; (C) ventral view; (D) ventral view of hand; (E) ventral view of foot. Photographs by Jinlong REN.

**Table 5** Measurements of *Raorchestes hillisi* sp. nov. from Xiding, Menghai, Yunnan (in mm).

Number	CIB 116329	CIB 116330	CIB 116331	CIB 116332	CIB 116333	CIB 116334	CIB 116335	Range	Mean ± S.D.	Ratio to SVL	CIB 116336	Ratio to SVL
Sex	♂	♂	♂	♂	♂	♂	♂			♀		
Status	Holotype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype			Paratype		
SVL	17.3	16.5	17.7	14.5	16.8	15.9	16.7	14.5–17.7	16.4 ± 1.1		17.5	
HL	7.3	7.3	8.1	6.1	6.4	6.1	6.3	6.1–8.1	6.9 ± 0.8	41.8%	7.1	40.6%
HW	6.5	6.0	6.5	5.1	6.0	5.7	6.1	5.1–6.5	5.9 ± 0.5	36.3%	6.6	37.9%
SL	2.6	2.4	2.7	2.5	2.5	2.6	2.6	2.4–2.7	2.5 ± 0.1	15.5%	2.8	15.9%
INS	2.0	1.9	1.9	1.6	1.7	1.7	1.8	1.6–2.0	1.8 ± 0.1	10.9%	1.7	9.7%
IOS	2.1	2.1	2.0	2.1	2.1	2.2	2.2	2.0–2.2	2.1 ± 0.1	13.0%	2.3	13.0%
UEW	1.5	1.5	1.6	1.1	1.3	1.3	1.3	1.1–1.6	1.3 ± 0.2	8.2%	1.5	8.6%
EHD	2.3	2.2	2.2	2.0	2.1	2.1	2.1	2.0–2.3	2.1 ± 0.1	13.0%	2.1	11.9%
TD	0.8	0.7	0.9	0.7	0.8	0.7	0.9	0.7–0.9	0.8 ± 0.1	4.7%	0.9	4.9%
FAHL	9.0	8.4	9.1	7.2	7.9	7.2	8.6	7.2–9.1	8.2 ± 0.8	49.9%	8.7	49.4%
LAW	1.7	1.8	1.8	1.1	1.5	1.6	1.4	1.1–1.8	1.5 ± 0.2	9.3%	1.2	7.0%
HAL	4.9	4.8	5.1	4.3	4.5	4.5	4.6	4.3–5.1	4.7 ± 0.3	28.4%	4.7	26.9%
FML	8.5	7.6	8.4	7.2	7.5	6.7	7.7	6.7–8.5	7.6 ± 0.6	46.6%	7.5	42.7%
TBL	8.3	7.6	8.4	7.2	7.6	7.5	7.9	7.2–8.4	7.8 ± 0.4	47.6%	8.1	46.3%
TFL	11.3	10.2	11.5	8.9	10.4	9.9	10.7	8.9–11.5	10.4 ± 0.9	63.2%	11.1	63.5%
FOL	6.7	6.1	6.8	5.6	6.2	5.6	6.1	5.6–6.8	6.2 ± 0.5	37.8%	6.6	37.7%

**Figure 6** Photographs of individuals of *Raorchestes hillisi* sp. nov. from the type locality, Xiding, Menghai, Yunnan, showing (A) dorsolateral view of a male in the wild; (B) dorsolateral view of a female indoors; (C) a calling male in the wild; (D) a pair of individuals preparing amplexus indoors. Photographs by Kai WANG.



**Figure 7** Photographs of holotype of *Raorchestes hillisi* sp. nov., showing (A) dorsal view; (B) ventral view; (C) ventral view of hand; (D) ventral view of foot. Photographs by Junfeng GUO.

from Southeast Asia and southern China except for this new species, including *R. cangyuanensis*, *R. gryllus* (Smith, 1924), *R. longchuanensis*, *R. menglaensis*, and *R. parvulus* (Frost, 2019; Yu et al., 2019). The new species differs from *R. cangyuanensis* by smaller body size (males SVL 15.9–17.7 mm,  $n = 7$  vs. 16.1–20.0 mm,  $n = 3$ ), head longer than wide, tympanum distinct, outer metatarsal tubercle present, discs of fingers and toes not orange (vs. males SVL 16.1–20.0 mm,  $n = 3$ , head wider than long, tympanum indistinct, outer metatarsal tubercle absent, discs of fingers and toes orange); differs from *R. gryllus* by having a head longer than wide, tympanum distinct, toe webbing rudimentary, and lacking a series of tubercles along the outer sides of forearm and foot (vs. head wider than long, tympanum indistinct, toe webbing more than half, and having a series of tubercles along the outer sides of forearm and foot); differs from *R. longchuanensis* by head longer than wide, fingers lacking lateral dermal fringe, discs of fingers and toes not orange (vs. head length equal to width, fingers I and II having lateral dermal fringes, discs of finger and toes orange); differs from

*R. menglaensis* by head longer than wide, tympanum distinct, dorsal surface smooth, without white tubercles (vs. head length equal to width, tympanum indistinct, dorsal surface rough, with white tubercles); differs from *R. parvulus* by head longer than wide, snout longer than eye diameter, canthus rostralis distinct, and fingers lacking lateral dermal fringe (vs. head wider than long, snout shorter than eye diameter, canthus rostralis rounded, and inside of all fingers having lateral dermal fringe).

***Raorchestes huanglianshan* sp. nov.** Jiang, Wang, Ren, and Li (Figures 9, 10).

*Kirtixalus menglaensis*: Yu, Rao, Zhang, and Yang, 2009, Mol. Phylogenet. Evol., 50: 578.

*Pseudophilautus menglaensis*: Li, Che, Murphy, Zhao, Zhao, Rao, and Zhang, 2009, Mol. Phylogenet. Evol., 53: 519.

*Raorchestes menglaensis*: Yu, Liu, Hou, Li, and Yang, 2019, Zootaxa, 4577 (2): 386.

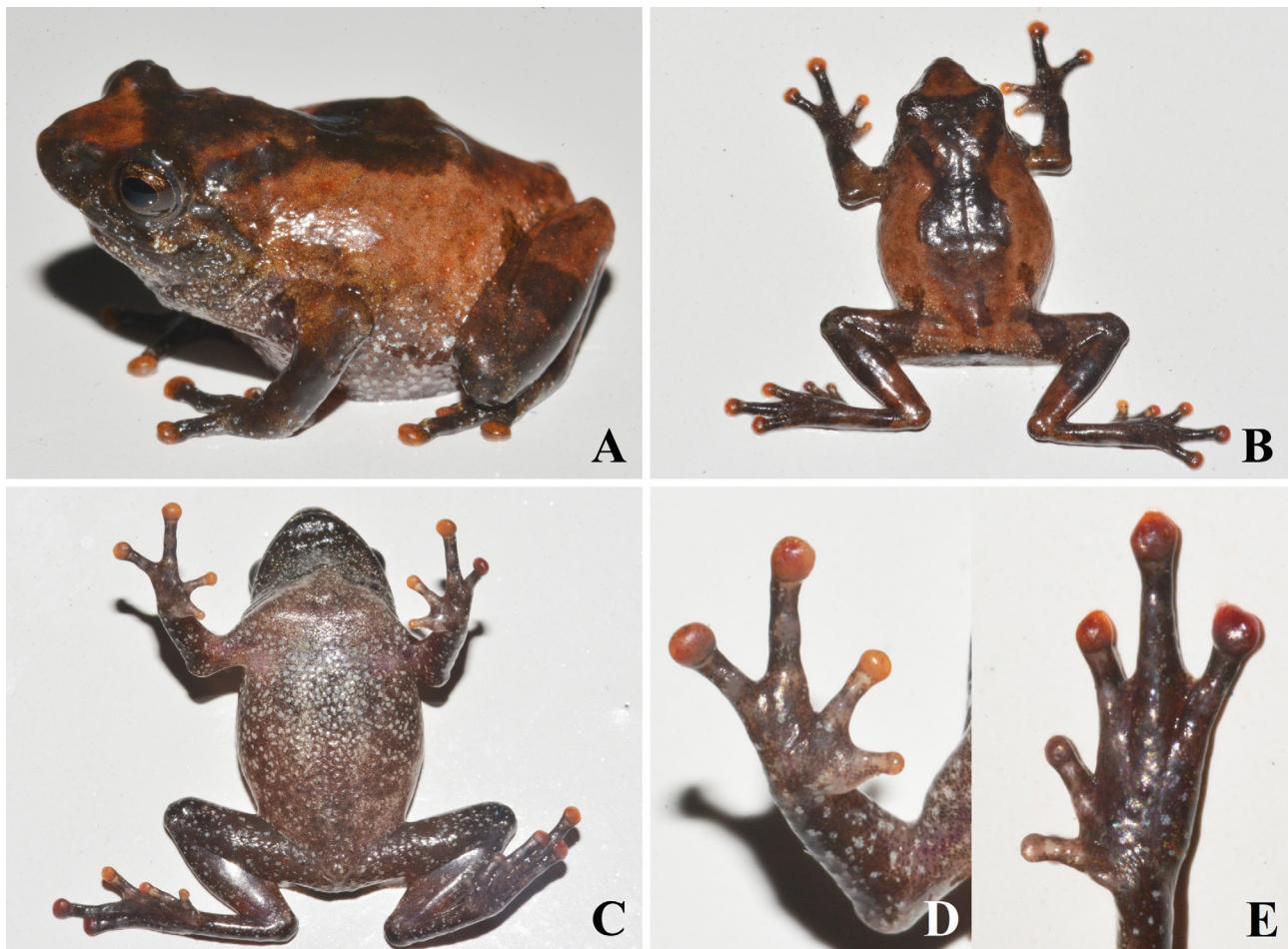
**Holotype:** an adult male CIB 116353 (field no. YN2018303),

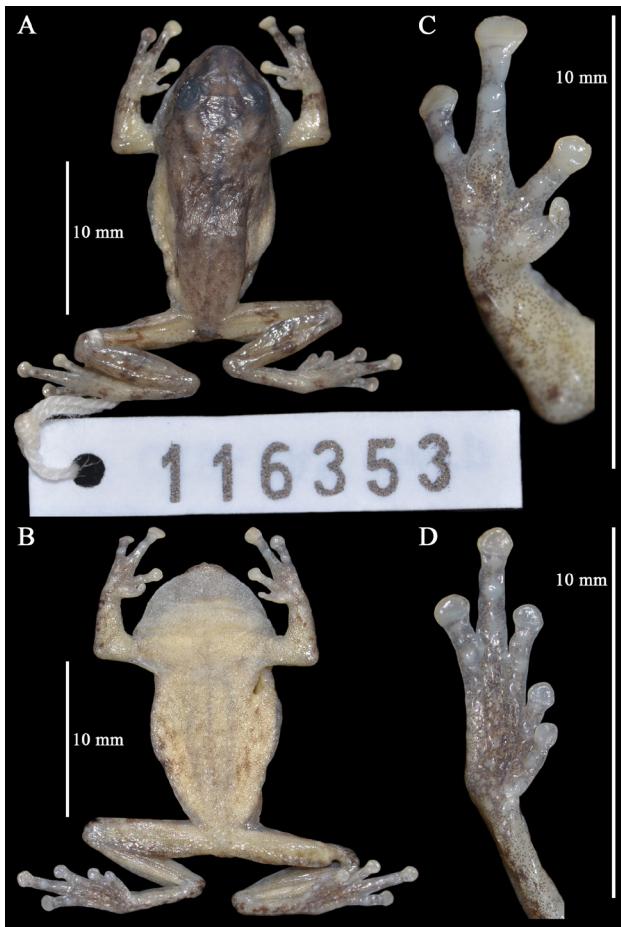


**Figure 8** Habitat of (A) *Raorchestes hillisi* sp. nov. from the type locality, Xiding, Menghai, Yunnan, altitude 1719 m, and (B) *Raorchestes huanglianshan* sp. nov. from the type locality, Mt. Huanglian, Lvhun, Yunnan, altitude 1823 m. Photographs by Ke JIANG and Jian WANG.

**Table 6** Measurements of *Raorchestes huanglianshan* sp. nov. from Mt. Huanglian, Luchun, Yunnan (in mm). P: Paratype, H: Holotype.

Number	CIB 116353	CIB 116354	CIB 116355	CIB 116356	CIB 116357	CIB 116358	CIB 116359	CIB 116360	CIB 116361	CIB 116362	CIB 116363	CIB 116364	Range	Mean ± S.D.	Ratio to SVL	CIB 116365	Ratio to SVL
Sex	♂	♂	♂	♂	♂	♂	♂	♂	♂	♂	♂	♂	♂		♀		
Status	H	P	P	P	P	P	P	P	P	P	P	P	P		P		
SVL	19.6	18.0	17.0	17.9	19.0	19.0	17.7	17.3	17.6	17.8	18.3	18.8	17.0–19.6	18.2 ± 0.8		21.5	
HL	7.3	6.5	6.4	6.5	6.8	6.9	6.4	6.2	6.4	6.2	6.3	7.0	6.2–7.3	6.6 ± 0.3	36.3%	8.3	38.6%
HW	7.4	6.7	6.5	6.5	6.8	6.9	6.7	6.4	6.5	6.3	6.3	7.0	6.3–7.4	6.7 ± 0.3	36.8%	8.4	38.9%
SL	3.1	2.8	2.6	2.7	2.8	2.9	2.8	2.8	2.6	2.7	2.6	2.7	2.6–3.1	2.8 ± 0.1	15.2%	3.2	14.8%
INS	2.1	2.0	2.0	2.0	1.9	2.2	1.9	2.0	1.9	2.0	1.9	1.9	1.9–2.2	2.0 ± 0.1	11.0%	2.4	11.3%
IOS	2.6	2.6	2.4	2.8	2.7	2.6	2.7	2.3	2.5	2.2	2.7	2.6	2.2–2.8	2.6 ± 0.2	14.0%	3.0	14.0%
UEW	1.6	1.6	1.6	1.3	1.4	1.5	1.4	1.4	1.3	1.4	1.4	1.6	1.3–1.6	1.5 ± 0.1	8.0%	1.8	8.2%
EHD	2.4	2.4	2.4	2.2	2.1	2.4	2.3	2.4	2.3	2.2	2.3	2.7	2.1–2.7	2.3 ± 0.2	12.8%	3.0	14.1%
TD	1.1	0.8	0.9	1.0	1.0	1.2	0.8	1.0	0.9	0.9	0.8	1.0	0.8–1.2	0.9 ± 0.1	5.2%	0.8	3.6%
FAHL	10.1	9.1	8.6	9.2	9.3	9.2	8.8	9.1	9.0	8.8	9.2	9.2	8.6–10.1	9.2 ± 0.4	50.4%	9.8	45.6%
LAW	1.7	1.6	1.6	1.4	1.7	2.1	1.7	1.4	1.6	1.6	1.7	1.6	1.4–2.1	1.6 ± 0.2	9.1%	1.5	6.9%
HAL	5.8	5.0	5.2	5.2	5.2	5.6	5.1	5.1	5.0	5.2	5.2	5.2	5.0–5.8	5.2 ± 0.2	28.8%	5.6	26.1%
FML	8.5	8.3	7.4	8.0	8.1	8.2	8.2	8.8	8.1	7.3	8.4	8.4	7.3–8.8	8.1 ± 0.4	44.7%	9.3	43.0%
TBL	8.6	8.1	7.7	8.1	8.2	8.0	7.8	8.2	8.0	6.9	8.3	8.4	6.9–8.6	8.0 ± 0.4	43.8%	9.5	44.4%
TFL	12.4	11.5	11.2	11.1	11.5	12.2	11.4	11.2	10.8	10.8	11.5	11.5	10.8–12.4	11.5 ± 0.5	63.0%	13.4	62.5%
FOL	7.3	7.1	6.5	6.8	6.8	7.7	7.0	6.9	6.6	6.8	6.7	7.0	6.5–7.7	6.9 ± 0.3	38.2%	8.5	39.6%

**Figure 9** Photographs of a male individual of *Raorchestes huanglianshan* sp. nov. from the type locality, Mt. Huanglian, Luchun, Yunnan, showing (A) dorsolateral view; (B) dorsal view; (C) ventral view; (D) ventral view of hand; (E) ventral view of foot. Photographs by Jinlong REN.



**Figure 10** Photographs of holotype of *Raorchestes huanglianshan* sp. nov., showing (A) dorsal view; (B) ventral view; (C) ventral view of hand; (D) ventral view of foot. Photographs by Ke JIANG.

collected from Mt. Huanglian (22.932255°N, 102.288264°E, altitude 1823 m), Lvchun, Yunnan, China on May 2018 by Jian WANG.

**Paratypes:** 11 adult males (CIB 116354–364), and an adult female (CIB 116365), as same locality and date as holotype, collected by Jian WANG.

**Etymology:** the specific epithet “*huanglianshan*” is named after the type locality Mt. Huanglian, “shan” means “mountain” in Chinese. We suggest “Huanglianshan Bush Frogs” as its English common name, and “Huang Lian Shan Guan Shu Wa ( 黄连山灌树蛙 )” as its Chinese common name.

**Diagnosis:** this new species is identified to the genus *Raorchestes* by its molecular phylogenetic position and the following morphological characters: (1) having small body size (SVL 15.0 to 45.0 mm); (2) large gular pouch transparent while calling; (3) lacking vomerine teeth.

*Raorchestes huanglianshan* sp. nov. is distinguished from all other congeners by the following combination of morphological

characters: (1) small body sized (males 17.0–19.6 mm,  $n = 12$ ; female 21.5 mm,  $n = 1$ ); (2) head slightly wider than long or equal; (3) snout longer than eye diameter; (4) the tip of upper jaws slightly notched; (5) internasal space distinctly less than interorbital space (6) tympanum distinct; (7) fingers lacking lateral dermal fringe; (8) toes lacking lateral dermal fringe; (9) rudimentary web on toes; (10) lacking a series of tubercles along the outer sides of forearm and foot; (11) discs of all fingers and toes orange or parts of them orange in life.

**Description of holotype:** small body size (SVL = 19.6). Head width near equal to head length (HW/HL = 1.01); snout rounded, upper jaw protruding slightly in ventral view; the tip of upper jaws slightly notched; canthus rostralis distinct; loreal region concave; snout slightly longer eye diameter (SL/ED = 1.29); nostrils oval, lacking flap, closer to snout tip than to eye; internasal space less than interorbital space (IOS/INS = 1.24); interorbital space 1.63 times larger than upper eyelid width (IOS = 2.6 mm; UEW = 1.6 mm); tongue notched at the tip; lingual papillae absent; vomerine teeth absent; tympanum distinct; supratympanic fold distinct; eyes small, pupil horizontal; a large external single subgular vocal sac present, a pair of slit-like openings near the inner corners of mouth.

Forelimb length near equal to half of body length (FAHL/SVL = 0.52); fingers lacking lateral dermal fringe and web; subarticular tubercles prominent (fingers: I = 1, II = 1, III = 2, IV = 1); relative lengths of fingers: I < II < IV < III; discs rounded; circummarginal grooves present on all fingers; nuptial pad present on the dorsal surface of first finger.

Hindlimbs short, heels just touch when folded at right angles to the body (TBL = 8.6 mm, FML = 8.5 mm); tibiotarsal articulation reaching the eye when adpressed; supernumerary tubercles and subarticular tubercle present (toes: I = 1, II = 1, III = 2, IV = 3, V = 2); relative lengths of toes: I < II < III < V < IV; disks rounded, the size of disks equal to these on fingers; circummarginal grooves present on all toes; no web between toe I and toe II, web rudimentary between other toes, webbing formula: II 1-2 III 1-2 IV 2-1 V; toes lacking lateral dermal fringe; subarticular tubercles prominent, rounded; inner metatarsal tubercle distinct, oval, outer metatarsal tubercle absent.

Skin on dorsal surface smooth, large tubercles scattered on back. Large tubercles scattered on the posterior temporal region and lateral body; supratympanic fold distinct. Ventral surface covered with large flat tubercles.

**Coloration in life:** dorsal color brownish-gray, with a brown triangular blotch on interorbital space, two patches of orange pigmentation on dorsal head; an indistinct “X” shaped brown blotch on back, from posterior eyes to middle of back; a brown blotch on each crotch; each side of dorsal thigh and tibia with one distinct brown bars; both dorsal and ventral side of discs

orange, rather distinct on ventral side. Three brown spots under the eye, and a brown stripe under the supratympanic fold. Ventral sides of head and body grayish-white, with small gray spots, the tubercles white; ventral side of limbs gray, with white spots. Iris brown.

**Coloration in preservative:** dorsal color changed to pale brownish-gray, the blotches or spots brownish-gray. Ventral color yellowish-white, spots brownish-gray.

**Variation:** The measurements are given in Table 6 and the variation are as follows: CIB 116359 having a pale line along the middle of back and hind limb; two males (CIB 116363–64) having distinctly darker ground color on dorsal side.

**Sexual dimorphism:** in males, white nuptial pad present on the dorsal surface of the first finger; a large external single subgular vocal sac present, a pair of slit-like openings near the inner corners of mouth; white lineae masculinae visible on ventral body; the body size of males less than the female.

**Distribution:** currently known from the type locality, Mt. Huanglian (Figure 4), Lvchun, Yunnan, China. Based on the phylogenetic relationships, it is probably also from Chiangmai, Thailand.

**Habitat:** this species was found calling from April to October on low plants near evergreen broad-leaved forest in Huanglianshan Nature Reserve, at relatively high altitude 1600–1900 m (Figure 8). Large numbers of individuals were observed on the leaves of *Ageratina adenophora* (Spreng.) in October after raining, and courtship behavior was also observed, the temperature was 14–20°C. In the habitat, more than ten amphibian species were found, such as: *Tylocotriton shanjiang* Nussbaum, Brodie, and Yang, *Leptobrachella ventripunctatus* (Fei, Ye, and Li), *Megophrys jingdongensis* Fei and Ye, *Megophrys rubrimera* Tapley, Cutajar, Mahony, Chung, Dau, Nguyen, Luong, and Rowley, *Hyla gongshanensis* Li and Yang, *Nidirana lini* (Chou), *Odorrana jingdongensis* Fei, Ye, and Li, *Nanorana yunnanensis* (Anderson), *N. unculuanus* (Liu, Hu, and Yang), *Feihyla fuhua* Fei, Ye, and Yang, *Gracixalus yunnanensis* Yu, Hui, Wang, Rao, Wu, and Yang, *Zhangixalus feae* (Boulenger), *Rhacophorus rhodoporus* Liu and Hu.

**Comparison:** *Raorchestes hillisi* sp. nov. differs from *R. cangyuanensis* by snout longer than eye diameter, tympanum distinct, outer metatarsal tubercle present, discs of fingers and toes not orange (snout shorter than eye diameter or equal, tympanum indistinct, outer metatarsal tubercle absent, discs of fingers and toes orange); differs from *R. gryllus* by tympanum distinct, toe webbing rudimentary, and lacking a series of tubercles along the outer sides of forearm and foot (vs. tympanum indistinct, toe webbing more than half, and having a series of tubercles along the outer sides of forearm and foot);

differs from *R. longchuanensis* by wider than long, fingers lacking lateral dermal fringe, dorsal body having a distinct “X” shaped black blotch (vs. head length equal to width, fingers I and II having lateral dermal fringes, dorsal body lacking a “X” shaped black blotch or indistinct); differs from *R. menglaensis* by head wider than long, tympanum distinct, dorsal surface smooth, without white tubercles, discs of fingers and toes orange (vs. head length equal to width, tympanum indistinct, dorsal surface rough, with white tubercles, discs not orange); differs from *R. parvulus* by snout longer than eye diameter, canthus rostralis distinct, and fingers and toes lacking lateral dermal fringe (vs. snout shorter than eye diameter, canthus rostralis rounded, and inside of all fingers having lateral dermal fringe, toes having lateral dermal fringe except for toe V); differs from *R. hillisi* by head wider than long, toes lacking lateral dermal fringe, discs of fingers and toes orange (vs. head longer than wide, toes having lateral dermal fringe except outside of toe I and both sides of toe II, discs not orange).

#### 4. Discussion

**4.1. Taxonomic confusions in *Raorchestes menglaensis***  
Taxonomic studies of *Raorchestes* are difficult to be conducted due to their morphological conservativeness and remarkably similar characters, which may cause ambiguities in taxonomy and distributions. The record of *R. parvulus* from China was reported by Yu et al. (2019) based on specimens collected from Menglun, Mengla, southern Yunnan, but these samples clustered with our *R. menglaensis* from the type locality (Zhushihe, Mengla, Yunnan) without distinct divergence (Figure 1; Table 2). Furthermore, the important morphological character of the specimens provided by Yu et al. (2019) is distinctively different from *R. parvulus* (Boulenger, 1893; Bossuyt and Dubois, 2001) in having both fingers and toes lacking lateral dermal fringes (vs. fingers and toes having fringes, but not evident on toe V in *R. parvulus*) whereas similar to *R. menglaensis*. Thus, based on our results, we conclude that the record of *R. parvulus* from China that reported by Yu et al. (2019) is actually misidentification of *R. menglaensis*, and it should be revised to *R. menglaensis*.

Moreover, Yu et al. (2019) used a sequence of *R. menglaensis* from GenBank (GenBank accession no. GQ285676) in the phylogenetic tree and the locality was given as “Mengla, Yunnan”. However, according to the GenBank accession no. (GQ285676), this sequence was submitted by Li et al. (2009) and the locality was actually provided as “Lvchun, Yunnan”, and this sequence clustered with our samples of *R. huanglianshan* from Lvchun, Yunnan. Therefore, the sequence of *R. menglaensis* (GQ285676) used by Yu et al. (2019) should be revised to *R. huanglianshan*.

#### 4.2. Taxonomic identity of *Raorchestes parvulus* *Raorchestes*

*parvulus* was originally described by Boulenger (1893) from “Karin Bia-po”, eastern Myanmar (Figure 4; orange circle, no. 3). This species has been widely reported across northern Indochina, including Myanmar, China, Thailand, Vietnam, Laos, Cambodia, Bangladesh and peninsular Malaysia (Frost, 2020), however, most of the country records lacking detailed comparison with the type specimen and original description (Bourret, 1942; Taylor, 1962; Nutphund, 2001; Ohler *et al.*, 2002; Stuart *et al.*, 2005; Grismer *et al.*, 2006; Ghose and Bhuiyan, 2012). Additionally, recent phylogenetic analysis failed to support the monophyly of *R. parvulus* clade (Chan *et al.*, 2018; Wu *et al.*, 2019), and none of the available sequences of *R. parvulus* in GenBank is come from its type locality, which means the phylogenetic position of *R. parvulus* cannot be confirmed at present.

According to the original description of *R. parvulus* provided by Boulenger (1893) and description of lectotype (MSNG 29838.A, adult female) by Bossuyt and Dubois (2001), it can be distinguished from all Chinese congeners by the following combination of morphological characters: (1) head broader than long; (2) snout length shorter than eye diameter; (3) interorbital space larger than upper eyelid, equal to internarial distance; (4) tympanum distinct; (5) dermal fringe on in side of all fingers; (6) dermal fringe on all toes, but not evident along toe V. Therefore, *R. parvulus* is currently unknown from China. Consequently, the record of *R. parvulus* should be delisted from Chinese herpetofauna.

**4.3. Underestimated *Raorchestes* diversity from China**  
Yet despite the fact that few *Raorchestes* species have been known from China, the recent studies continuously uncovered *Raorchestes* diversity from China (Wu *et al.*, 2019; this study). Along with the two new species described herein, the number of the genus *Raorchestes* is increased to five, including *R. longchuanensis*, *R. menglaensis*, *R. cangyuanensis*, *R. hillisi*, and *R. huanglianshan*. Due to the small body size and limited dispersal ability, *Raorchestes* species could be easily affected by biogeographic isolation, which may generate considerable biodiversity. These recent findings suggest that the true *Raorchestes* biodiversity from China has been underestimated, and future herpetofauna survey will likely continue to yield new discoveries from the region.

**Acknowledgements** We thank Mr. Kai WANG (University of Oklahoma, USA), Mr. Lingui HE, Mr. Wenbo YU, Mr. Zhongxiong FU, Mr. Hanliu AI, Mr. Guangxu HUANG, and Mr. Wen ZHANG for the fieldwork, Mr. Zhian ZHANG (Huanglianshan National Nature Reserve) for supporting our research, Mr. Mian HOU (Sichuan Normal University) for proving some information. We also thank the reviewers for their suggestions. This work was supported by the Strategic Priority Research Program of Chinese Academy

of Sciences (CAS) (XDB31000000); the National Natural Science Foundation of China (31722049, 31900322, 31401983); the CAS “Light of West China” Program (2018XBZG\_JCTD\_001) and Talent Program from Organization Department of Sichuan Provincial Committee; Biological Resources Programme, Chinese Academy of Sciences (KFJ-BRP-017-14), the International Partnership Program of Chinese Academy of Sciences (151751KYSB20190024), and Biodiversity Survey, Observation and Assessment Program of Ministry of Ecology and Environment of China and Honghe University (14bs10, 2015HB0302) to WJ.

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Handling Editor: Chen YANG

**How to cite this article:**

**Jiang K., Ren J. L., Wang J., Guo J. F., Wang Z., Liu Y. H., Jiang D. C., Li J. T.** Taxonomic Revision of *Raorchestes menglaensis* (Kou, 1990) (Amphibia: Anura), with Descriptions of Two New Species from Yunnan, China. *Asian Herpetol Res*, 2020, 11(4): 263–281. DOI: 10.16373/j.cnki.ahr.200018